Protocol for Labeling DNA by Random Oligonucleotide-primed Synthesis (with Klenow Fragment, exo-)

1. Dissolve 100ng of DNA in 10µl of water.
2. Add:
   - 10X reaction buffer 5µl,
   - 12.5 A_{260}units/ml random decamer primer or
     7.5 A_{260}units/ml random hexamer primer 10µl,
   - deionized water to 40µl.
3. Incubate the mixture in a boiling water bath for 5-10 minutes and then cool immediately on ice.
4. Add:
   - 0.33mM 3dNTP mix (without labeled dNTP) 3µl (0.02mM - final concentration),
   - [alfa-^{32}P]-dNTP (3000Ci/mmol) 50µCi,
   - Klenow fragment, exo- 5u,
   - deionized water up to 50µl.
5. Incubate the reaction mixture with random decamer primer at 37°C for 5 minutes and with hexamer primer for 10 minutes.
6. Add 4µl 0.25mM dNTP mix and incubate the reaction mixture at 37°C for 5 minutes.
7. Stop the reaction by the addition of 1µl 0.5M EDTA (pH 8.0).
8. Remove 1µl of the reaction mixture and determine the percentage of label incorporated.

Reference

1. Feinberg, A.P., Vogelstein, B., Addendum to: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity, Anal. Biochem., 137, 266-267, 1984.